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PRINCIPAL INVESTIGATOR: Gail P. Risbridger, Ph.D.

CONTRACTING ORGANIZATION: Monash University
Clayton, Victoria 3168
Australia

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Gail P. Risbridger, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Monash University
Clayton, Victoria 3168
Australia

E-Mail: Gail.risbridger@med.monash.edu.au

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13. ABSTRACT (Maximum 200 Words)

This project aims to identify adult prostate stem cells, using tissue recombination technology. To date, we have successfully directed the differentiation of mouse and human embryonic stem (ES) cells as outlined in the original statement of work. We have shown pathologically and histologically that the resultant tissue recombinants are highly characteristic of mouse and human prostate. We have refined the technology so that we reliably get prostate-like tissues in the absence of spontaneous differentiation of ES cells into non-prostate like tissues. We are currently restrained by the technology associated with the transfection of human embryonic stem cells, but hope to complete all the tasks of the project during 2005.

This work has been presented at a total of 6 meetings including both national meetings within Australia internationally in the USA. We plan to publish the initial findings early in 2005. Work is ongoing to tag and isolate epithelial cells from these tissues in order to identify the adult prostate stem cell.

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Table of Contents

INTRODUCTION

This project ultimately aims to identify adult stem cells in the prostate gland. It is well accepted that stem cells reside within the prostate epithelium, and it has been suggested that they are housed within the basal cell compartment. However, to date, prostate stem cells have not been isolated or characterized. We believe that the stem cells in the adult prostate have a major role to play in the initiation and progression of prostate carcinogenesis and so identifying these cells will have major implications in treating prostate cancer. We are employing a unique approach involving the use of mouse and human embryonic stem cells to track the differentiation of prostate epithelial cells to identify adult prostate stem cells.

BODY

Task 1: To generate prostatic ductal structures from mouse ES cells using tissue recombination techniques (months 1-6).

As reported in last year's annual report, we have completed all the experimental aims listed under task 1. To date, these results remain unpublished.

Task 2: To use these tissues for the isolation of sufficient numbers of basal and intermediate cell types from the epithelia, that can be tagged with appropriate markers prior to differentiation (months 6 – 18).

As reported last year, we have made some progress towards this task. We have successfully generated two constructs that will be used to transfect mouse and/or human ES cells prior to recombination in preparation for cell sorting following the grafting period (task 3).

The first construct is the human keratin 18 gene promoter driving DsRed expression and the second is the human keratin 5 gene promoter driving GFP expression. These constructs are available for use as soon as the appropriate transfection technology can be employed.

The reason this aim has not progressed is based on technical difficulties. The technology for transfecting human embryonic stem cells is in its infancy and we are not yet in a position to conduct the required experimental steps to tag and isolate specific epithelial cells. Hopefully with rapid progress in the field, we will be able to complete this task during 2005. In the absence of being able to transfect human embryonic stem cells, we will attempt transfections with mouse embryonic stem cells where the technology is much further advanced. This is not ideal since we are mostly interested in human ES cells, but it will be sufficient to fulfill our project aims.

Task 3: To determine which cell type is the prostatic stem cell of the epithelia (months 12-24)

As reported last year, we have not made any progress on this task to date since we have not yet successfully transfected ES cells with our tagging constructs. Once we achieve that aim, we will embark on tracking prostate epithelial differentiation using the methods described in this task.

Task 4: To adopt the same strategies and techniques to prove the identity of human prostate stem cells using human ES cells (months 24-36).

As we reported last year, although we originally stated that we would not undertake these experiments until months 24-36 of the funding, we initiated these studies within the first year and have made significantly more progress during the second year of funding.

We have refined the recombination protocol so that we can reliably produce prostate-like tissues. We have significantly reduced the incidence of non-prostate-like structures that arise in recombinants as a result of spontaneous differentiation of human embryonic stem cells. By controlling the differentiation of human embryonic stem cells, we have produced tissues that are pathologically similar to adult human prostate (Figure 1).

A section of adult human prostate is shown in Figure 1A. Pathologically, human prostate is composed of glandular ductal structures which are embedded in a mass of fibromuscular stroma (Figure 1A). Tissues generated from tissue recombination of human embryonic stem cells and prostatic mesenchyme are morphologically similar to human prostate (Figure 1A compared to Figure 1B, C, D).

In addition to the pathological similarities, the prostate generated from ES cells shows highly characteristic immunolocalisation of androgen receptors. (see Figure 1B, C, D). Androgen receptors are highly expressed in both the epithelium and surrounding stromal cells. Interestingly, human embryonic stem cells do not express androgen receptors in their undifferentiated state. As a result of tissue recombination, these cells have become androgen-responsive, as you would expect of human prostate tissue.

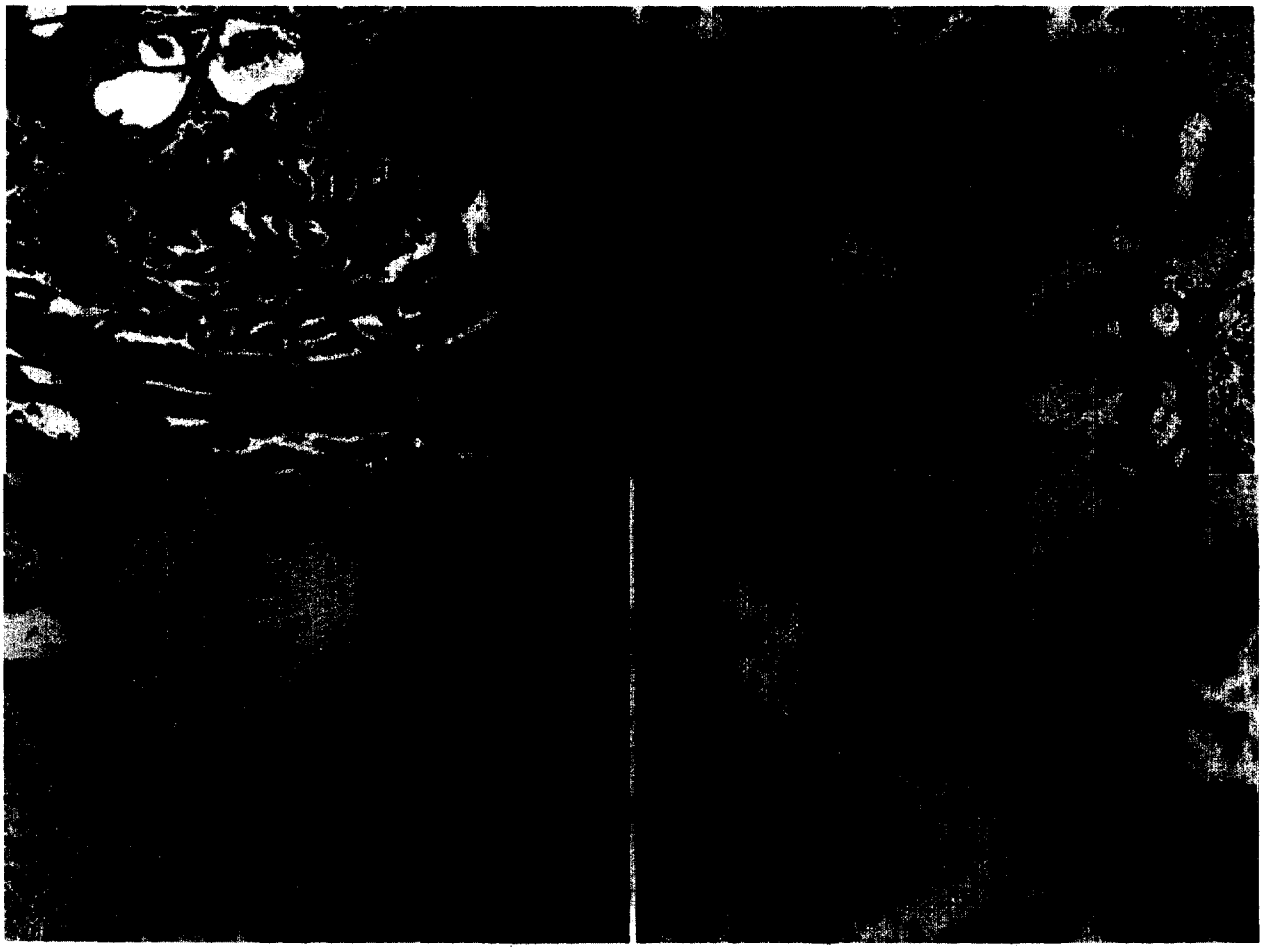

Figure 1 – Human prostate pathology compared to tissue generated from human embryonic stem cells. a. *H&E stained section of human prostate.* ***b-d.*** *Prostate-like tissues generated from human embryonic stem cells as* ***b.*** *x10,* ***c.*** *x20 and* ***d.*** *x40.*

In addition to morphological characteristics, we have analysed the tissue using characteristic cellular markers to demonstrate appropriate patterning within both the epithelium and surrounding stroma.

Firstly, we have demonstrated appropriate cytodifferentiation within the epithelium. Using cytokeratin high-molecular weight as an epithelial cell marker, immuno-positive basal cells were evident at the basal aspect of the epithelial layer, whilst an immuno-negative secretory epithelial cell layer is evident at the luminal aspect of the epithelium (Figure 2A). The ratio of basal:secretory epithelial cells is what you would expect in human prostate epithelium. Based on these markers, we seem to have replicated the normal prostate epithelial cell progeny which is critical to the success of the project as we intend to sort the different epithelial cell subtypes and identify the adult prostate stem cell.

In addition, we have characterized the cellular components in the stroma. Smooth muscle α-actin is appropriately expressed around the ductal structures (Figure 2B). There are pockets of immuno-negative stromal cells that are most likely fibroblasts or other components of the stromal network including vasculature, lymphatics or nervous fibers.

Figure 2. – Epithelial and stromal cell markers in prostate tissue generated from human embryonic stem cells. *a. Immunolocalisation of cytokeratin high molecular weight (epithelial cell marker).* ***b.*** *Smooth muscle α-actin (stromal cell marker).*

Based on pathological and morphological analysis, the prostate tissue generated from human embryonic stem cells is highly characteristic of human prostate. However, in order to provide definitive evidence that the tissues we have generated are in fact human prostate, we would like to see the expression of the common prostate secretory marker, prostate-specific antigen (PSA). To our surprise, the recombinant tissues are consistently immuno-negative for PSA expression (Figure 3A), compared to human prostate (Figure 3B) which strongly expresses the secretory protein.

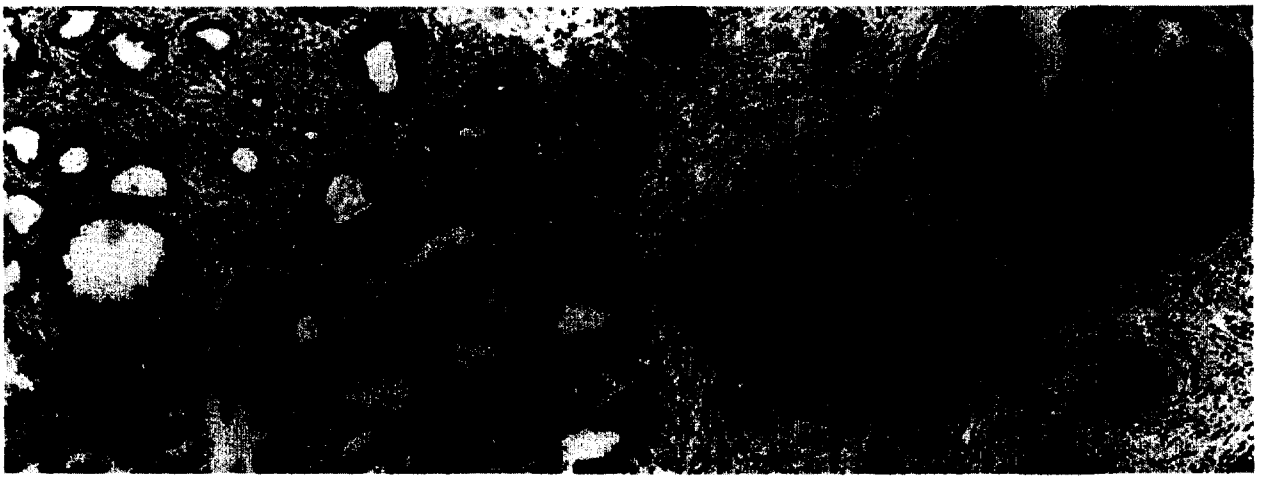

Figure 3. – Immunlocalisation of prostate-specific antigen. *a. Prostate tissue generated from human embryonic stem cells. b. Adult human prostate tissue.*

We do not have an explanation for this; it is an extremely puzzling finding for both us and our colleagues since we are so confident that we are producing is human prostate by all other hallmarks. We are currently working on finding other prostate-specific markers to prove this tissue is prostate.

Once we get definitive proof of the tissue-type, we will put all this data together for publication (expected submission date April 2005). In order to complete the project, we will then tackle the tagging and isolation of epithelial cell types as described in tasks 2 and 3.

KEY RESEARCH ACCOMPLISHMENTS

List of key research accomplishments emanating from this research:

- Proved concept of controlled differentiation of ES cells using both SVM and UGM.
- Directed differentiated mouse embryonic stem cells into prostate-like tissues.
- Directed differentiated human embryonic stem cells into prostate-like tissues and fully characterized tissues by pathological and morphological analysis.
- Generated constructs for CK18 and CK 5 for future transfections.

REPORTABLE OUTCOMES

	Reportable outcomes that have resulted form this research:
Manuscripts	Nil (in preparation)
Abstracts Presentations	1. **Taylor RA**, McPherson SJ, Ellem SJ, Frydenberg M, Risbridger GP (2005) All power to the prostatic stroma. *Annual Scientific Meeting of the Urological Society of Autraliasia*, Melbourne, Australia. *(Poster presentation)* 2. **Taylor RA**, Cunha GR, Trounson AO, Risbridger GP (2005) Stromal microenvironment influences stem cell differentiation in normal and malignant prostate. *17th Lorne Cancer Conference*, Phillip Island, Australia. *(Oral presentation)* 3. **Jarred RA**, Wang H, Trounson AO, Risbridger GP (2004) Directed differentiation of human embryonic stem cells into prostate using tissue recombination. *The 86th Annual Meeting of the Endocrine Society*, New Orleans, USA. **(#This abstract was selected for inclusion in Endo Newsline television program and was awarded travel grant from the Endocrine Society as well as the Australian Women in Endocrinology DSL New Investigator Travel Award;** *poster presentation***)** 4. **Jarred RA**, Wang H, Trounson AO, Risbridger GP (2004) Directed differentiation of human embryonic stem cells into prostate using tissue recombination. *The 2nd International Society for Stem Cell Research Conference*, Boston, USA. *(Poster presentation)*
Patents and licences	Nil.
Degrees obtained	Nil.
Development of cell lines	Nil.
Tissue or serum repositories	Nil.
Informatics such as databases and animal models	Nil.
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Employment or research	Nil.

opportunities applied for and/or received based on experience/training supported by this award	

CONCLUSIONS

In summary, we have made significant progress towards directing differentiation of mouse and human ES cells into prostate lineages. We have shown pathologically and histologically that the resultant tissue recombinants have several characteristics of mouse and human prostate. Specifically, we have refined the technology to reliably produce human prostate-like tissues. This model will now be useful to identify the adult prostate stem cell.

This progress will have major implications to our understanding of prostate biology. In the same way that differentiation of embryonic stem cells into neurons has advanced the neurological field, these findings will have a major impact on urology. This research will further our understanding of the factors that induce prostate differentiation, that have eluded us until now.

REFERENCES

N/A

APPENDIX 1: ABSTRACT PRESENTATIONS

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1. **Taylor RA**, McPherson SJ, Ellem SJ, Frydenberg M, Risbridger GP (2005) All power to the prostatic stroma. *Annual Scientific Meeting of the Urological Society of Australiasia*, Melbourne, Australia. *(Poster presentation)*

ALL POWER TO THE PROSTATIC STROMA

Renea A. Taylor, Stephen J. McPherson, Stuart J. Ellem, Mark Frydenberg, Gail P. Risbridger

[1]*Monash Institute of Reproduction and Development, Monash University, Clayton, Victoria, Australia*

Reciprocal interactions between stroma and epithelia are critical for normal development and function of the prostate gland. Disruption of this relationship is believed to give rise to prostate diseases including PCa and BPH. Elucidation of the biological mechanisms involved in stroma-tumor interactions could provide significant new advances in therapeutic interventions. Tissue recombination is based on the ability of embryonic urogenital or seminal vesicle mesenchyme (UGM or SVM respectively) to act as permissive and instructive inductors of epithelium which allows detailed analysis of the effects of the prostatic microenvironment on prostate growth and development. Tissue recombinants of normal mouse SVM and human embryonic stem cells (hESCs) or aromatase knockout (ArKO) SVM and normal mouse epithelium were grafted under the kidney capsule of adult male immune-deficient mice thereby allowing them to develop in an adult male hormonal environment for 4-12 wks before pathological and immunohistochemical analysis. Under the influence of normal prostatic mesenchyme, hESCs differentiated into glandular structures pathologically similar to normal human prostate with androgen receptor-positive glands lined by pseudostratified epithelial cells expressing characteristic cytokeratin profiles. However ArKO tissue recombinants demonstrated induction of hyperplasia in the previously normal epithelium as a direct result of the influence of ArKO mesenchyme. In combination, these studies demonstrate the power of prostatic mesenchyme to dictate the fate of adjacent epithelium. In addition, these studies support the hypothesis that the prostatic stroma may be a potential target for therapeutic interventions for prostate diseases.

2. **Taylor RA,** Cunha GR, Trounson AO, Risbridger GP (2005) Stromal microenvironment influences stem cell differentiation in normal and malignant prostate. *17th Lorne Cancer Conference*, Phillip Island, Australia. *(Oral presentation)*

STROMAL MICROENVIRONMENT INFLUENCES STEM CELL DIFFERENTIATION IN NORMAL AND MALIGNANT PROSTATE

Taylor RA[1], Trounson AO[2], Cunha GR[3], Risbridger GP[1]

[1]Monash University, Melbourne, Victoria, Australia (Center for Urology Research)
[2]Monash University, Melbourne, Victoria, Australia (Monash Immunology and Stem Cell Laboratories)
[3]Department of Anatomy, University of California, San Francisco CA

The reciprocal interactions between stroma and epithelia of the prostate are critical for the development and normal function of the prostate gland. Prostate cancer is believed to arise from the sequential disruption of these interactions. In order to develop a novel model system to explore the biological mechanisms of prostate development and malignant transformation, we developed a system to differentiate human epithelial cells *in vivo*. In this study, human embryonic stem cells (hESCs) were used as a renewable reproducible source of epithelial cells. Directed differentiation of hESCs was conducted using tissue recombination. Briefly, hES2 and hES4 cells (ES Cell International, Singapore) were grafted under the kidney capsule of adult male SCID mice for 4, 8 and 12 weeks alone or in combination with neonatal mouse prostate mesenchyme, after which time tissues were collected for pathological and immunohistochemical analysis. When grafted alone hESCs for teratocarcinomas, whereas when grafted in combination with neonatal prostate mesenchyme, grafts consisted of ductal structures that were histologically indistinguishable from immature human prostate. Androgen receptor was expressed in stromal and epithelial cells, characteristic of human prostate. After 12 weeks, grafts showed signs of maturation as evident by ductal glands lined by a psueudostratified columnar epithelium composed of a full complement of epithelial cells as well as lumen formation and evidence of secretory products. The surrounding stroma was organised in a multilayered concentric ring around the cords indicating the reciprocal nature of the interactions between the stroma and epithelia during differentiation. Therefore, we successfully directed the differentiation of hESCs into prostate epithelium *in vivo*. This model system is a reproducible reliable means to study stroma-stem cell interactions in normal and malignant tissues. In addition, it provides a novel screening tool to identify therapeutic agents that target the tumor microenvironment

3. **Jarred RA**, Wang H, Trounson AO, Risbridger GP (2004) Directed differentiation of human embryonic stem cells into prostate using tissue recombination. *The 86th Annual Meeting of the Endocrine Society,* New Orleans, USA. (***This abstract was selected for inclusion in Endo Newsline television program and was awarded travel grant from the Endocrine Society as well as the Australian Women in Endocrinology DSL New Investigator Travel Award;** *poster presentation*)

DIRECTED DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO PROSTATE USING TISSUE RECOMBINATION

Renea A. Jarred[1], Hong Wang[1], Alan Trounson[2] and Gail P. Risbridger[1]
[1]*Monash Institute of Reproduction and Development, Monash University, Clayton, Victoria, Australia*
[2]*National Stem Cell Centre, Notting Hill, Victoria, Australia*

The prostate gland is a male reproductive organ that is located at the base of the bladder and is often associated with disease upon aging, specifically prostate cancer and benign prostatic hyperplasia. In order to study the cell differentiation lineage associated with normal and diseased prostate, we used human embryonic stem (hES) cells as a tool to initially generate normal human prostate using tissue recombination. Tissue recombination utilizes the inductive and instructive potential of prostate mesenchyme to direct differentiation of hES cells in to prostate epithelial cells. Briefly, hES2 cells (ES Cell International; Reubinoff *et al.*, 2000) were recombined with neonatal mouse seminal vesicle mesenchyme (SVM) and grafted under the kidney capsule of adult male immune-deficient mice for periods of up to 8 weeks. The tissue was collected and prepared for pathological and immunohistochemical analysis. The resultant grafted tissue was prostate-like, consisting of secretory glands lined by a psuedostratified columnar secretory epithelium, embedded in fibromuscular stroma. Using expression markers, we confirmed the epithelium was of human origin using a human-specific antibody raised to cytokeratins 8 & 18 (epithelial cell markers). In addition, the tissue was immuno-positive for androgen receptors, both in the stromal and epithelial cell types, consistent with prostate expression pattern. This study provides the first evidence of directed differentiation of hES cells into human prostate tissue. The long term aims of this project are to label the hES cells prior to recombination in order to trace the differentiation pathway of human prostate epithelial cells in the hope of identifying adult human prostate stem cells.

Reference: Reubinoff BE, Pera MF, Fong C-F, Trounson AO, Bongso A. Embryonic stem cell lines form human blastocysts: somatic differentiation in vitro. Nat Biotech 2000, 18: 399-404.

4. **Jarred RA**, Wang H, Trounson AO, Risbridger GP (2004) Directed differentiation of human embryonic stem cells into prostate using tissue recombination. *The 2nd International Society for Stem Cell Research Conference,* Boston, USA. *(Poster presentation)*

DIRECTED DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO PROSTATE USING TISSUE RECOMBINATION

Renea A. Jarred[1], Hong Wang[1], Alan Trounson[2] and Gail P. Risbridger[1]
[1]*Monash Institute of Reproduction and Development, Monash University, Clayton, Victoria, Australia*
[2]*National Stem Cell Centre, Notting Hill, Victoria, Australia*

Pluripotent embryonic stem (ES) cells are the most versatile cells, with the potential to differentiate into all types of cells. Much research is currently focused on techniques that can control differentiation of ES cells to specific cell lineages. In this study, we are attempting to differentiate human embryonic stem (hES) cells into prostate tissue using tissue recombination techniques. The prostate gland is a male reproductive organ that is located at the base of the bladder and is often associated with disease upon aging, specifically prostate cancer and benign prostatic hyperplasia. In order to study the cell differentiation lineage associated with normal and diseased prostate, we utilized the inductive and instructive potential of prostate mesenchyme to direct differentiation of hES cells in to prostate epithelial cells. Briefly, hES2 cells (ES Cell International; Reubinoff *et al.*, 2000) were recombined with neonatal mouse prostate mesenchyme (seminal vesicle mesenchyme or urogenital mesenchyme) and grafted under the kidney capsule of adult male immune-deficient mice for 2 weeks, 8 weeks or 12 weeks. The tissue was collected and prepared for pathological and immunohistochemical analysis. The resultant grafted tissue was prostate-like, consisting of secretory glands lined by a psuedostratified columnar secretory epithelium, embedded in fibromuscular stroma. Using expression markers, we confirmed the epithelium was of human origin using a human-specific antibody raised to cytokeratins 8 & 18 (epithelial cell markers). In addition, the tissue was immuno-positive for androgen receptors, both in the stromal and epithelial cell types, consistent with prostate expression pattern. This study provides the first evidence of directed differentiation of hES cells into human prostate tissue. The long term aims of this project are use this unlimited supply of human prostate tissue to identify adult human prostate stem cells that may potentially be targeted for prostate disease treatments in the future.

Reference: Reubinoff BE, Pera MF, Fong C-F, Trounson AO, Bongso A. Embryonic stem cell lines form human blastocysts: somatic differentiation in vitro. Nat Biotech 2000, 18: 399-404.